pathways, but when combined with electrophysiological recordings, potentially also the computations that these pathways embody.

Future Research on Forelimb Movements

The new studies on the role of MdV in grasping [3] and on that of V2a-PN in reaching types of movements [4] beautifully highlight how deeply functional topographical principles are embedded in the brain, even when these are not directly evident from the cyto-architecture and even when they are studied in lower mammals like mice. As these building blocks are now becoming more apparent, the obvious question that arises is how these different phases of forelimb movements, resulting from different muscle activities and different control centers in the brainstem and spinal cord, are coordinated over time. Undoubtedly, the olivocerebellar system, which is readily accessible with genetic approaches using cell-specific promoters, plays a pivotal role in this coordination [19,20]. By showing the diverse viral and optogenetic applications as well as the precise functional topography for forelimb movements, the Arber and Jessell labs are acting as guides to the main functional questions on coordination control, both in terms of technical approaches and the concrete neuro-anatomical targets in the brainstem and spinal cord that need to be investigated.

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Evolution: Hidden at the End of a Very Long Branch

DNA-based methods continue to unveil the diversity and evolutionary origins of life on Earth. 'Next generation' methods have just solved a long-standing puzzle by uncovering previously unseen yet globally distributed diversity within a lineage of amitochondriate parasites affecting commercially exploited aquatic hosts. This discovery will impact both pure and applied research fields.

Cathryn L. Abbott

Mikrocytos mackini is a mysterious microbe that causes Denman Island disease in Pacific oysters (*Crassostrea gigas*) on the northwest coast of North America. The disease causes mortality in oysters as well as unsightly green lesions which result in reduced marketability [1,2]. It is perplexing to consider how a parasite described in 1988 and now known to represent a unique amitochondriate lineage completely eluded the scientific literature on eukaryotic evolution until last year [3,4]. Amitochondriate eukaryotic lineages are rare, and have been salient to empirical studies of early eukaryotic evolution since 1983 when Cavalier-Smith formalized the now defunct theory that they comprise a primitive eukaryotic group (Archezoa) that evolved before the endosymbiotic origin of the mitochondrion [5]. The fact is *M. mackini* is astoundingly elusive. It is among the tiniest eukaryotes (Figure 1), has no defining morphological features, had no known relatives (until now), occurs in only one part of the world, is not culturable, has an unknown life cycle, and disappears for part of the year because the disease it causes is temperature-dependent [6]. '*Mikrocytos*-like' organisms have been reported from various parts of the world but the identity of the parasites could not be confirmed nor the detections repeated (e.g., [7–9]).

Figure 1. *Mikrocytos mackini*.
A histological section of Pacific oyster tissue heavily infected with *Mikrocytos mackini* (arrows). (Image kindly provided by Gary R. Meyer, Nanaimo, BC.)

Indeed, it has proven very difficult to answer any of the big scientific questions that would 'blow open' our understanding of this enigmatic parasite. Mercifully, two studies published in *Current Biology*, one in this issue from Hartikainen *et al.* [10] and an earlier one from Burki *et al.* [4] (in collaboration with current author), have done just that. The evolutionary origins of *Mikrocytos* have now been elucidated and the genus has a home within a new and potentially highly diverse taxonomic order of parasites called Mikrocytida [10].

Hartikainen *et al.* [10] used next generation sequencing to uncover a wealth of previously unknown diversity within mikrocytids. So, where was this diversity hiding while other amitochondriate lineages occupied the limelight of eukaryotic evolutionary research during the rise and subsequent fall of the Archezoan hypothesis? The answer is: pretty much everywhere. Hartikainen *et al.* [10] found them in freshwater, brackish, and marine environments from five continents and infecting hosts from across three invertebrate phyla. So, if they were everywhere, why did we not find them? It is because, phylogenetically speaking, they were hidden at the end of a very long branch.

Variation in the rate of molecular evolution of DNA is reflected in varying branch lengths in phylogenetic trees; faster-evolving taxa occur at the ends of longer branches. Intuition says discerning an entity at the end of a long branch would be easy; however, not when dealing with phylogenetic branches (Figure 2). Fast-evolving lineages are prone to being artificially placed at the base of phylogenetic trees due to an analytical artefact called long branch attraction [11,12]. This leads to their being misinterpreted as primitive relative to other taxa. Amitochondriate lineages are characteristically long branched, and for a time the long branch attraction problem generated false support for the now disproven Archezoan hypothesis [11].

Sure enough, mikrocytids have evolved at such an extremely high rate that they are in fact one of the most divergent eukaryotic lineages [4,10]. Indeed, the first attempt to find the evolutionary origins of *M. mackini* was foiled by long branch attraction [13]. Fortunately, better evolutionary models and more data are making it easier to accurately place long branches [11]. Burki *et al.* [4] convincingly established *Mikrocytos* as a member of the eukaryotic supergroup Rhizaria despite its branch being several-fold longer than most other branches on the tree (Figure 2). Hartikainen *et al.* [10] have now followed with an elegant complement to the Burki *et al.* [4] study. Burki *et al.* [4] located *Mikrocytos* within Rhizaria by maximizing gene sampling; they used a phylogenomic data set comprising 119 genes. Hartikainen *et al.* [10] used 4 genes but maximized taxon sampling to resolve the phylogenetic position of mikrocytids within Rhizaria: they are the sister group of haplosporidians. They used a rigorous sequence signature analysis to verify the accuracy of their phylogeny, ensuring against misinterpretation stemming from long branch artefacts [10].

The extreme evolutionary rate of mikrocytids explains how a globally distributed and potentially highly diverse lineage remained undiscovered until now. DNA-based methods are very effective for detecting parasites and uncharacterized diversity. Available information on known diversity is used to design methods of capturing all diversity. It turns out that mikrocytid DNA evolves so quickly that these parasites are sufficiently genetically

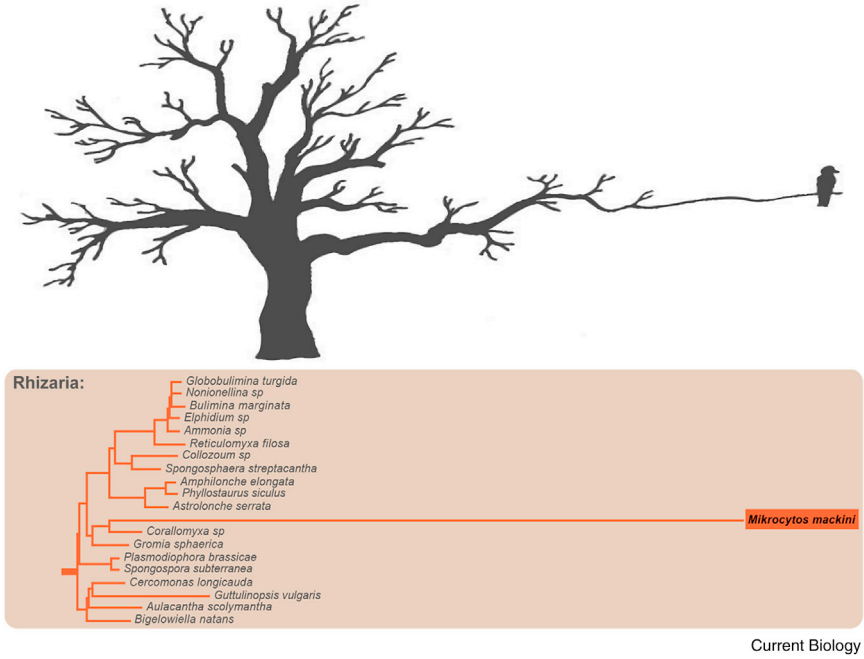

Figure 2. An extremely divergent lineage.
This image shows two long branches; one on an oak tree (top) and one on a phylogenetic tree belonging to an astoundingly fast-evolving oyster parasite, *Mikrocytos mackini* (bottom; adapted from Burki *et al.* [4]). It is intuitive that an organism at the end of a really long branch would be easy to find, but this is not so for phylogenetic branches. Extremely high evolutionary rates leading to high divergence from all other known eukaryotes explains why the diversity of mikrocytid parasites has eluded scientists until now. (Sketch kindly provided by Dr. Scott R. Gilmore, Nanaimo, BC.)

divergent from all other known organisms they were never detected by 'universal' methods and so were absent from public DNA databases [10]. Even levels of genetic variation within mikrocytids are so high that the DNA-based method of detecting *M. mackini* [13] did not lead to the jackpot of hidden diversity Hartikainen *et al.* [10] found by using new massively parallel sequencing technology.

High evolutionary rates in mikrocytids may be due to strong selection associated with reductive evolution. This is a process of extreme specialization to parasitic life. The cell structure, function, and genome size of the parasite become simplified as it adapts to rely on host cellular machinery instead of its own [14]. This was suggested for *M. mackini* over a decade ago based on its cell ultrastructure [15], and the discovery by Burki *et al.* [4] of a reduced mitochondrion-related organelle in *Mikrocytos* confirms it. It is now clear that mitochondria or related organelles are essential to all eukaryotic cells [14]. Comparative studies among phylogenetically independent amitochondriate lineages, including mikrocytids, could shed light on the limits of reductive evolution as well as lineage-specific innovations [14].

Hartikainen *et al.* [10] developed a genetic method specifically for finding mikrocytids that could lead to an explosion of new discoveries. Understanding mikrocytid diversity and distributions and what factors control their occurrence and spread is important for practical reasons. First, mikrocytids infect commercially important hosts. *M. mackini* is already a regulated disease in some countries. It was listed by the World Organization for Animal Health (OIE) but was de-listed in 2007. This decision may ultimately need to be revisited. The two new species described by Hartikainen *et al.* [10] were both found causing pathology in hosts that are harvested for human consumption: *Mikrocytos mimicus* n. sp. in Pacific oysters and *Paramikrocytos canceri* n. gen, n. sp. in the European edible crab (*Cancer pagurus*) [10]. Over the last decade, the Food and Agriculture Organization of the United Nations reports annual global aquaculture production of Pacific oysters as exceeding 600,000 tonnes and wild capture production of European edible crabs as usually exceeding 40,000 tonnes. Second, parasites are easily spread by the movement of their infected hosts and can be more destructive to naïve hosts than their usual ones. Indeed, lab studies have shown that oyster species other than Pacific oysters are more susceptible to the disease caused by *M. mackini* [16]. Also noteworthy here is that Hartikainen *et al.* [10] found *P. canceri* detections to be common in the European green crab (*Carcinus maenas*), one of the world's most notorious marine invasive species. It has invaded five continents and causes substantial negative impacts to recipient ecosystems, including significant reductions in abundances of native invertebrates [17,18]. An improved understanding of host specificity, pathogenicity, and degree of endemism of mikrocytids will help us evaluate risks associated with their introduction into naïve ecosystems.

Global diversity of protists is exceptionally high relative to that of multicellular eukaryotes [19]. Microsporidians are relatives of fungi that are outwardly similar to mikrocytids; they are also amitochondriate obligate intracellular parasites. There are over 1,200 described species of microsporidians [20]. Now that mikrocytids are no longer hidden from science, one can only wonder: how many will we find?

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